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Systematic Review and Meta-Analysis of Antigen Detection Tests for the Diagnosis of Tuberculosis▽†

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Tests that detect *Mycobacterium tuberculosis* antigens in clinical specimens could provide rapid direct evidence of active disease. We performed a systematic review to assess the diagnostic accuracy of antigen detection tests for active tuberculosis (TB) according to standard methods and summarized test performance using bivariate random effects meta-analysis. Overall, study quality was a concern. For pulmonary TB (47 studies, 5,036 participants), sensitivity estimates ranged from 2% to 100% and specificity from 33% to 100%. Lipoarabinomannan (LAM) was the antigen most frequently targeted (23 studies, 49%). The pooled sensitivity of urine LAM was higher in HIV-infected than HIV-uninfected individuals (47%; 95% confidence interval [CI], 26 to 68% versus 14%; 95% CI, 4 to 38%); pooled specificity estimates were similar: 96%; 95% CI, 81 to 100% and 97%; 95% CI, 86 to 100%, respectively. For extrapulmonary TB (21 studies, 1,616 participants), sensitivity estimates ranged from 0% to 100% and specificity estimates from 62% to 100%. Five studies targeting LAM, ESAT-6, Ag85 complex, and the 65-kDa antigen in cerebrospinal fluid, when pooled, yielded the highest sensitivity (87%; 95% CI, 61 to 98%), but low specificity (84%; 95% CI, 60 to 95%). Because of the limited number of studies targeting any specific antigen other than LAM, we could not draw firm conclusions about the overall clinical usefulness of these tests. Further studies are warranted to determine the value of LAM detection for TB meningitis in high-HIV-prevalence settings. Considering that antigen detection tests could be translated into rapid point-of-care tests, research to improve their performance is urgently needed.

The World Health Organization (WHO) estimates that in 2009, 9.4 million new cases of tuberculosis (TB) occurred and 1.7 million people died of the disease (89). The vast majority of these patients live in low- and middle-income countries (LMIC) where TB diagnosis depends primarily on smear microscopy. Microscopy has low sensitivity and does not detect smear-negative TB (77), which may account for 24% to 61% of all pulmonary cases in HIV-infected individuals (27). Improved diagnostic tests, such as mycobacterial culture and nucleic acid amplification (NAA) tests, are available in high-income countries but are often too expensive and complex for routine use by TB control programs in resource-constrained settings in which TB is endemic. Lack of access to diagnostic services in resource-limited settings presents an additional barrier to using these tests. The Xpert MTB/RIF (Cepheid, Inc., Sunnyvale, CA), recently endorsed by the WHO, is rapid and highly sensitive for detection of TB and drug resistance; however, this new technology is costly, preventing its use in many areas where the epidemic is most severe (10). Accurate, rapid, inexpensive, and simple diagnostic tests are urgently needed for TB care and control.

There have been considerable efforts over the past 50 years to devise a rapid TB test based on antibody detection. However, substantial published evidence suggests that current commercial serological tests are inaccurate for diagnosis of active TB (22, 73–76, 91). A meta-analysis of noncommercial serological tests identified several potential candidate antigens and antigen combinations for inclusion in an antibody detection test for pulmonary TB (PTB) (73). Recently, serological profiling of the complete *Mycobacterium tuberculosis* proteome has been described (38). In July 2011, the WHO issued a policy statement against the use of commercial serological tests for the diagnosis of active pulmonary and extrapulmonary TB (EPTB) while stressing the importance of continued research on antibody-based detection tests and point-of-care tests (90).

Another approach aims to detect circulating mycobacterial antigens in clinical specimens such as serum, sputum, urine, cerebrospinal spinal fluid (CSF), and pleural fluid. A common design for antigen detection tests is the sandwich enzyme-linked immunosorbent assay (ELISA) technique, also called

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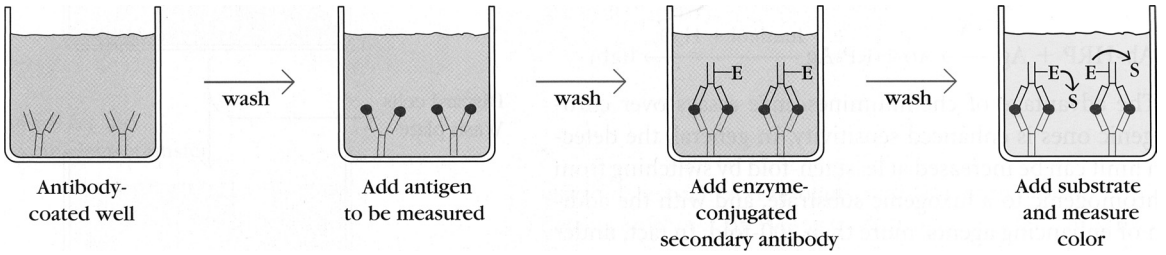

FIG. 1. Antigen-capture ELISA. (Far left) Antigen-specific monoclonal or polyclonal antibodies are coated onto the surface of plastic wells. (Second from left) The specimen containing the antigen to be measured is added. The antigen released is then "captured" by the antigen-specific antibodies. (Third from left) The captured antigen(s) are detected by secondary antibodies using the same antibody or an antibody directed toward an epitope that is different from the epitope recognized by the capture antibody. These secondary antibodies can be directly labeled with moieties, like biotin, or enzymes, like horseradish peroxidase. When biotin is used, visualization requires the addition of streptavidin conjugated to enzyme (horseradish peroxidase or alkaline phosphatase). (Far right) The appropriate substrate is added. The result is visualized by the color generated and measured with an ELISA plate reader. (Reprinted from reference 29a with permission of the publisher.)

the antigen-capture ELISA, described in Fig. 1. Another format is the lateral-flow immunochromatographic assay, sometimes referred to as a dipstick. In one common scheme of this technique, the antigen is initially introduced onto the device by adding a sample from a clinical specimen, such as urine or serum. Free antigen, if present, binds to an antibody/microsphere (bead) complex, providing a visually detectable color change (44). Dipsticks require little and sometimes no sample processing. Agglutination is an alternate method that can detect antigens when they clump together (agglutinate) with antibodies and form a visual complex.

In comparison with conventional diagnostics, antigen detection tests appear to offer several advantages. Immunochromatographic tests, for example, are rapid (results may be available within minutes) and easy to operate. If developed into a point-of-care test, an antigen detection test could extend TB diagnosis to remote health posts. Antigen detection tests provide direct evidence of active disease, thus allowing for immediate initiation of TB treatment. An antigen detection test using a specimen such as urine would be particularly attractive with children, who may have difficulty providing sputum. Finally, in patients suspected of extrapulmonary TB, an antigen detection test might prevent the use of more invasive tests.

To our knowledge, two systematic reviews have previously assessed the diagnostic accuracy of antigen detection tests for TB (22, 45). Both reviews reported on the performance of tests targeting lipoarabinomannan (LAM), a major glycolipid component of the cell wall of *M. tuberculosis* and other mycobacteria (12, 30). The first review identified one study evaluating sputum LAM; the sensitivity was 63%, and the specificity 92% (22). The second review focused on urine LAM (9 studies) (45). Depending on the classification of culture-negative clinical TB, pooled sensitivity estimates ranged from 34% to 60% and pooled specificity from 93% to 94%. Two recent narrative reviews were also identified (16, 55). However, one review focused only on urine-based tests (55), and neither review appraised study quality or performed a meta-analysis.

The objective of the current systematic review is to estimate the diagnostic accuracy of antigen detection tests using different clinical specimens for PTB and EPTB in adults and children with and without HIV infection.

MATERIALS AND METHODS

We followed methods for conducting and reporting systematic reviews and meta-analyses recommended by the Cochrane Collaboration Diagnostic Test Accuracy Working Group and the PRISMA statement, including the preparation of a protocol (26, 40, 46).

Selection criteria and definitions. (i) Types of studies. Included were diagnostic studies (with any study design) that evaluated antigen detection tests (using immunologic methods such as a sandwich ELISA) for PTB and EPTB in patients providing clinical specimens prior to or within 14 days of starting antituberculous treatment.

(ii) Participants. Participants included adults and children with suspected or confirmed active TB (at least 10 cases), with or without HIV infection.

(iii) Intervention (test). The tests consisted of an antigen detection test using an immunologic method, such as a sandwich ELISA or immunochromatographic assay.

(iv) Target conditions. Target conditions consisted of pulmonary TB and extrapulmonary TB.

(v) Reference standards. Positivity on culture was used for PTB and positivity on culture, smear, or histopathological examination for EPTB. For TB meningitis, we accepted positivity by commercial NAA tests because these tests provide high specificity (22, 51).

(vi) Outcomes. Sensitivity refers to the proportion of patients among TB patients with a positive antigen detection test result confirmed by the reference standard. Specificity refers to the proportion of non-TB participants with a negative antigen detection test result among non-TB participants. To estimate specificity, we selected only one non-TB group if a study had more than one such group. The preferred non-TB participants were those in whom active TB was initially suspected but later ruled out ("other respiratory disease" or "other disease" groups) and who were from the same population as TB patients.

EPTB was classified as lymph node, pleural, meningeal, and/or central nervous system, bone and/or joint, genitourinary, abdominal, skin, other site, disseminated, and multiple (extrapulmonary TB cases from different sites are combined to obtain at least 10 extrapulmonary TB cases).

Countries were classified as high income or LMIC according to the World Bank list of economies (88).

The following were excluded: (i) studies published before 1990, (ii) animal studies, (iii) conference abstracts and proceedings, (iv) studies of the detection of latent TB infection, (v) studies of the detection of nontuberculous mycobacteria, and (vi) basic science literature focused on the detection/cloning of new antigens or evaluation of their immunological properties (i.e., early preclinical studies).

Search methods. Database searching was performed (PubMed, 1 January 1990 to 16 April 2011, and Biosis, Embase, and Web of Science, 1 January 1990 to 10 February 2010) for relevant studies that reported data on immunological tests (based either on antigen or antibody detection) for active TB. For studies prior to 2006, databases were searched only in English because of limited resources, and for those after 2006, in all languages (see supplemental material S1). Reference lists of included papers and reviews were hand searched, and investigators contacted to identify additional studies.

Study selection. Initially, two reviewers (L.L.F. and K.R.S.) independently screened citations for relevance and then, based upon prespecified selection

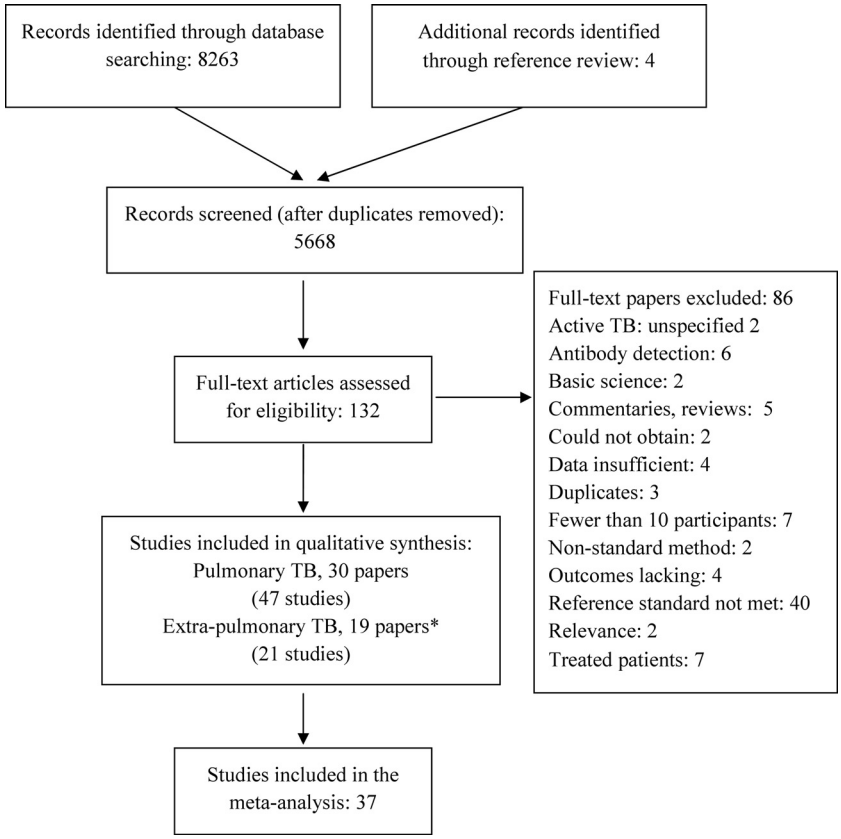

FIG. 2. Flow of studies in the review of antigen detection tests for the diagnosis of pulmonary and extrapulmonary TB. *, two of the papers in the extrapulmonary TB group are also included in the pulmonary TB group.

criteria, independently reviewed full-text articles. Disagreements about study selection were resolved by discussion between the reviewers.

Data extraction. A data extraction form was created, pilot tested with a subset of eligible studies, and then finalized. Two reviewers (L.L.F. and K.R.S.) independently extracted data from the included studies with the standardized form on the following characteristics: study design, age group (children < 15 years), HIV status, country, smear status, form of TB, assay type, test name, antigen identity, type of specimen, sensitivity, and specificity. When possible, studies were classified by HIV or smear status. Differences between reviewers concerned mainly methodological quality and were resolved by discussion. When necessary, authors were contacted for additional information.

We looked for information on patient-important outcomes, such as mortality, time to diagnosis, and number of patient visits during the diagnostic process.

Assessment of methodological quality. Two reviewers independently assessed study quality using QUADAS (Quality Assessment of Diagnostic Accuracy Studies), a validated tool to evaluate the presence of bias in diagnostic accuracy studies (86) (see supplemental material S2).

Data analysis. Descriptive analyses were performed using SPSS (version 14.0.1.366). For each study, the sensitivity and specificity of the test along with 95% confidence intervals (CI) were calculated, and forest plots created to display the estimates using Review Manager 5.1 (the Nordic Cochrane Center, Copenhagen, Denmark). Heterogeneity was assessed by visual examination of forest plots.

Selection of subgroups for meta-analysis. Heterogeneity was addressed by prespecifying subgroups by type of specimen evaluated, antigen(s) detected, and smear and HIV status (see Fig. S1 in the supplemental material). For the meta-analysis, at least four studies for inclusion in a subgroup were required.

Bivariate meta-analyses that jointly modeled both sensitivity and specificity were performed. These models weighted studies according to the sampling variability within studies as well as the unexplained heterogeneity between studies using a random effects approach (63). The model was estimated using a Bayesian approach with nonsubjective prior distributions and implemented using WinBUGS (version 1.4.1) (71). The meta-analysis provides a pooled estimate of the sensitivity and specificity across studies. A hierarchical summary receiver operating characteristic (HSROC) plot provides added information when there is high heterogeneity between studies, and the pooled estimate may not be representative of all studies. The HSROC curve plots sensitivity versus specificity and provides information on the overall performance of a test across different thresholds. The closer the curve is to the upper left hand corner of the plot (sensitivity and specificity are both 100%), the better is the performance of the test. The plots were made using R (version 2.6.1) (58).

RESULTS

Initially, 8,263 citations were identified (Fig. 2). After screening titles and abstracts, 132 potentially relevant full-text articles were retrieved; 49 publications describing a total of 68 studies (47 for PTB and 21 for EPTB) were selected for inclusion in the review. A list of excluded articles with reasons for exclusion is available upon request from the authors.

PTB. (i) Characteristics of included studies. Thirty papers, 29 in English (1, 2, 5, 6, 8, 9, 14, 15, 17–20, 23, 33, 37, 39, 43, 49, 50, 54, 57, 60, 61, 64, 66, 67, 70, 72, 85) (2 papers by Shah et al. concerned the same investigation) and one in French (24), were included (Table 1). The 47 PTB studies involved 5,036 participants (sample size = 6,540). The majority (81%) of the studies were conducted in LMIC. Eight studies involved HIV-infected individuals; none involved children. In each study, all non-TB participants were from the same country as TB patients. Twenty-three (49%) studies had a cross-sectional design, and 21 (45%) studies a case-control design. In three

TABLE 1. Characteristics of included studies evaluating antigen detection tests for the diagnosis of pulmonary tuberculosis[d]

Specimen type (no. of studies)	Author (reference) (year, study)[e]	Country	HIV status	Smear status	Comparison group	Test name/antigen(s) detected (H37Rv designation)	No. of patients with/without TB	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
Serum (12)	Chanteau (15) (2000, b)	Madagascar	HIV−	Smear+	ORD	In-house/45/47 kDa (1860)	64/23	28 (18–41)	96 (78–100)
	El-Masry (23) (2008)	Egypt	Not reported	Unspecified	ORD	In-house/20 kDa (not found)	175/65	91 (86–95)	89 (79–96)
	Kashyap (33) (2007)	India	HIV−	Smear+	OD	In-house/Ag85 complex (3804c and 1886c)	24/49	96 (79–100)	80 (66–90)
	Khomenko (37) (1996)	Russia	Not reported	Unspecified	ORD	In-house/*M. tuberculosis* antigens, unspecified	41/30	90 (77–97)	90 (73–98)
	Mamun (43) (1990, a)	Bangladesh	Not reported	Smear+	ORD	Latex Agglutination Test for TB/*M. tuberculosis* antigens, unspecified	18/26	94 (73–100)	77 (56–91)
	Mamun (43) (1990, b)	Bangladesh	Not reported	Smear−	ORD	Latex Agglutination Test for TB/*M. tuberculosis* antigens, unspecified	19/26	47 (24–71)	77 (56–91)
	Rajan (57) (2007)	India	Not reported	Smear+	OD	In-house/65 kDa (0440)	24/74	100 (86–100)	82 (72–90)
	Sada (64) (1992, a)	Mexico	HIV−	Smear+	ORD	In-house/LAM	50/63	88 (76–95)	100 (94–100)
	Sada (64) (1992, b)	Mexico	HIV+	Unspecified	ORD	In-house/LAM	21/63	57 (34–78)	100 (94–100)
	Sood (70) (1991)	India	Not reported	Smear+	Healthy	In-house/PPD	30/37	73 (54–88)	84 (68–94)
	Stavri (72) (1990)	Romania	Not reported	Smear+	OD	In-house/*M. tuberculosis* antigens, unspecified	42/201	79 (63–90)	75 (68–80)
	Verbon (85) (1993)	The Netherlands	Not reported	Unspecified	OD and healthy	In-house/38 kDa (0934)	44/120	48 (32–63)	97 (92–99)
Sputum (20)	al-Orainey (2) (1992, a)	Saudi Arabia	Not reported	Smear+	ORD	In-house/*M. tuberculosis* antigens, unspecified	29/176	83 (64–94)	95 (91–98)
	al-Orainey (2) (1992, b)	Saudi Arabia	Not reported	Smear−	ORD	In-house/*M. tuberculosis* antigens, unspecified	10/176	60 (26–88)	95 (91–98)
	Alavi-Naini (1) (2009, a)	Iran	HIV−	Smear+	ORD	Patho-TB/proprietary	43/111	93 (81–99)	70 (61–79)
	Alavi-Naini (1) (2009, b)	Iran	HIV−	Smear−	ORD	Patho-TB/proprietary	24/111	83 (63–95)	70 (61–79)
	Araj (5) (1993, a)	Kuwait	Not reported	Smear+	ORD	In-house/*M. tuberculosis* antigens, unspecified	24/45[a]	100 (86–100)	73 (58–85)
	Araj (5) (1993, b)	Kuwait	Not reported	Smear−	ORD	In-house/*M. tuberculosis* antigens, unspecified	47/45[a]	94 (82–99)	80 (65–90)
	Banchuin (6) (1990, a)	Thailand	Not reported	Smear+	ORD	In-house/PPD	69/164[a]	97 (90–100)	93 (88–97)
	Banchuin (6) (1990, b)	Thailand	Not reported	Smear−	ORD	In-house/PPD	16/164[a]	31 (11–59)	93 (88–97)
	Ben-Selma (8) (2009)	Tunisia	Not reported	Smear+	ORD	Patho-TB/proprietary	79/21	95 (88–99)	100 (84–100)
	Chakraborty (14) (2009)	India	HIV+	Unspecified	ORD	Diagnos TB Ag/LAM, 30 kDa (1886c) and 2 proprietary antigens	98/102	98 (93–100)	99 (95–100)
	Chanteau (15) (2000, a)	Madagascar	HIV−	Smear+	ORD	In-house/45/47 kDa (1860)	64/23	22 (13–34)	96 (78–100)
	Cho (17) (1997)	Korea	Not reported	Unspecified	ORD	In-house/LAM	24/38	63 (41–81)	92 (79–98)
	Deodhar (19) (1998, a)	India	Not reported	Smear+	ORD	In-house/19 (3763), 38 (0934), and 58 kDa (0440)	198/131	89 (84–93)	89 (82–93)
	Deodhar (19) (1998, b)	India	Not reported	Smear−	ORD	In-house/19 (3763), 38 (0934), and 58 kDa (0440)	116/131	82 (74–88)	89 (82–93)
	Dheda (20) (2010, c)	South Africa	HIV+	Unspecified	ORD	Clearview TB ELISA/LAM	44/41	100 (92–100)	7 (2–20)
	Dheda (20) (2010, d)	South Africa	HIV−	Unspecified	ORD	Clearview TB ELISA/LAM	80/109	84 (74–91)	16 (9–24)
	Fabre (24) (2007)[b]	France	Not reported	Unspecified	ORD	Patho-TB/proprietary	110/78	96 (91–99)	86 (76–93)
	Pereira Arias-Bouda (54) (2000)	Vietnam	Not reported	Unspecified	OD	In-house/LAM	18/21	94 (73–100)	100 (84–100)

	Reither (61) (2010, a)	Tanzania	HIV+	Unspecified	ORD	Diagnos TB Ag/LAM, 30 kDa (1886c) and 2 proprietary antigens	48/90	65 (49–78)	33 (24–44)
	Reither (61) (2010, b)	Tanzania	HIV−	Unspecified	ORD	Diagnos TB Ag/LAM, 30 kDa (1886c) and 2 proprietary antigens	28/90	50 (31–69)	33 (24–44)
Urine (15)	Boehme (9) (2005, a)	Tanzania	HIV±	Smear+	ORD	LAM-ELISA/LAM	82/82	83 (73–90)	90 (82–96)
	Boehme (9) (2005, b)	Tanzania	HIV±	Smear−	ORD	LAM-ELISA/LAM	50/82	76 (62–87)	90 (82–96)
	Daley (18) (2009, a)	India	HIV±	Smear+	ORD	LAM- ELISA/LAM	27/155	22 (9–42)	88 (82–92)
	Daley (18) (2009, b)	India	HIV±	Smear−	ORD	LAM- ELISA/LAM	21/155	52 (30–47)	88 (82–92)
	Dheda (20) (2010, a)	South Africa	HIV+	Unspecified	ORD	Clearview TB ELISA/LAM	44/41	20 (10–35)	100 (91–100)
	Dheda (20) (2010, b)	South Africa	HIV−	Unspecified	ORD	Clearview TB ELISA/LAM	80/109	6 (2–14)	99 (95–100)
	Lawn (39) (2009)[c]	South Africa	HIV+	Unspecified	ORD	LAM-ELISA/LAM	58/177	33 (21–46)	100 (98–100)
	Mutetwa (49) (2009, a)	Zimbabwe	HIV±	Smear+	ORD	LAM-ELISA/LAM	121/114	50 (41–60)	89 (81–94)
	Mutetwa (49) (2009, b)	Zimbabwe	HIV±	Smear−	ORD	LAM-ELISA/LAM	40/114	28 (15–44)	89 (81–94)
	Napolitano (50) (2008)	US and Brazil	Not reported	Unspecified	Healthy	In-house/(1656)	16/16	38 (15–65)	100 (79–100)
	Reither (60) (2009, a)	Tanzania	HIV−	Smear+	ORD	LAM-ELISA/LAM	17/45	24 (7–50)	91 (79–98)
	Reither (60) (2009, b)	Tanzania	HIV+	Smear+	ORD	LAM-ELISA/LAM	31/37	74 (55–88)	84 (68–94)
	Reither (60) (2009, c)	Tanzania	HIV+	Smear−	ORD	LAM-ELISA/LAM	19/37	42 (20–67)	84 (68–94)
	Shah (66) (2010, a)	South Africa	HIV±	Smear+	OD	Clearview TB ELISA/LAM	73/122	64 (52–75)	96 (91–99)
	Shah (66) (2010, b)	South Africa	HIV±	Smear−	OD	Clearview TB ELISA/LAM	88/122	53 (42–64)	96 (91–99)

[a] Unit of analysis is specimen rather than patient.
[b] Cases included both treated and untreated patients; results were nearly equivalent when stratified by treatment status.
[c] Specimen was unconcentrated urine.
[d] ELISA, enzyme-linked immunosorbent assay; LAM, lipoarabinomannan; OD, other diseases; ORD, other respiratory diseases; TB, tuberculosis; PPD, purified protein derivative; HIV±, people with and without HIV infection.
[e] Lowercase letters following the year of study indicate distinct studies, as some papers may have included more than one study.

TABLE 2. Antigen detection tests for the diagnosis of pulmonary TB

Name(s) of antigen(s) detected (H37Rv designation for protein antigens)	No. of studies	Reference(s)
Lipoarabinomannan	20	9, 17, 18, 20, 39, 49, 54, 60, 64, 66
Mycobacterium tuberculosis antigens, unspecified	8	2, 5, 37, 43, 72
Proprietary	4	1, 8, 24
Lipoarabinomannan, 30 kDa (1886), and 2 other proprietary	3	14, 61
Purified protein derivative (PPD)	3	6, 70
45/47 kDa (1860)	2	15
19 (3763), 38 (0934), and 58 kDa (0440)	2	19
20 kDa (Rv not found)	1	23
Ag85 complex (3804c and 1886c)	1	33
Rv1656	1	50
38 kDa (0934)	1	85
65 kDa (0440)	1	57

studies, study design was unclear. The median number of TB patients included in each study was 43 (interquartile range of 24 to 71).

Of the total 47 studies, 36 (77%) used ELISA, 7 (15%) an immunochromatographic test, and 4 an agglutination test. As shown in Table 2, single antigens were targeted in 24 (51%) studies and multiple antigens in 21 studies; the number of antigens was not reported in two studies. Of the 47 studies, LAM was the antigen most frequently targeted, either as a single antigen or one of several antigens (23 studies, 49%). Among the 20 studies targeting LAM alone, urine was the specimen most often evaluated (14 studies). In 15 (32%) studies, antigen identity was not reported. Five commercial tests were evaluated, accounting for 25 (53%) of the total studies: Patho-TB (Anda Biologicals, France; 4 studies), Diagnos TB Ag (Biomed Industries, India; 3 studies), LAM-ELISA (Chemogen, United States; 10 studies [this assay was a precommercial prototype and the test is no longer available]), Clearview TB ELISA (Inverness Medical Innovations, United States; 6 studies), and a latex agglutination test for TB (Wellcome Diagnostics, United Kingdom; 2 studies).

As assessed with QUADAS, all studies satisfied six quality items: acceptable reference standard (a criterion for inclusion in the review), acceptable delay between tests, partial verification avoided, incorporation avoided, reference standard results blinded (as the culture result was considered to be entirely objective in interpretation), and relevant clinical information. However, studies had serious shortcomings in two essential items, representative spectrum (selection of a representative patient population) and blinding, factors that have been shown empirically to be associated with exaggerated accuracy estimates (42): only 18 (38%) studies involved a representative patient population and only 19 (40%) reported blinding of the result of the antigen detection test (see Fig. S2 in the supplemental material).

Figure 3 is a forest plot of the sensitivity and specificity of

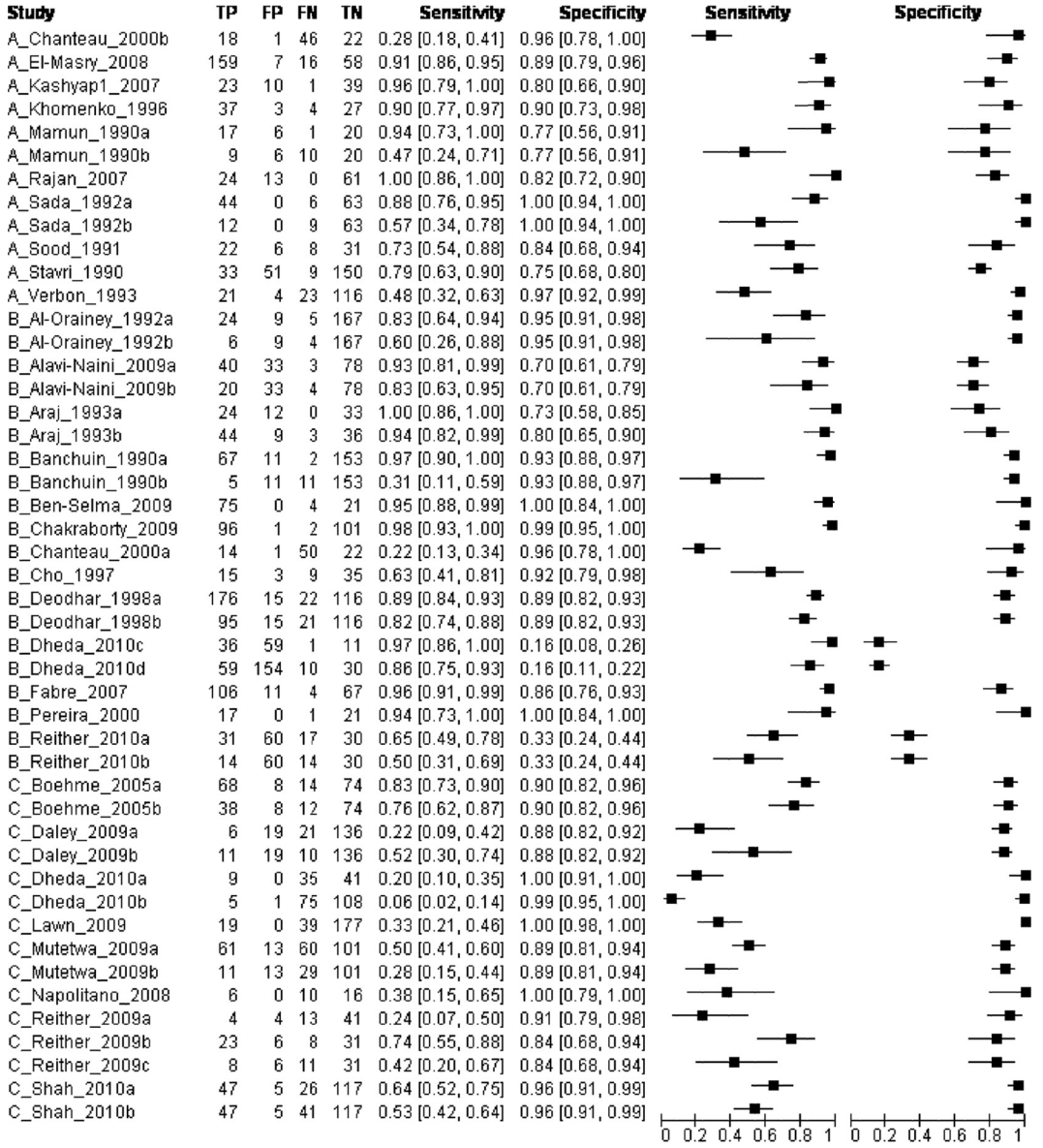

FIG. 3. Forest plots of sensitivity and specificity for antigen detection tests for pulmonary TB, all studies. For letters preceding author names: A, serum; B, sputum; C, urine. (Lowercase letters following year of study indicate distinct studies.) TP, true positive; FP, false positive; FN, false negative; TN, true negative. The 95% confidence intervals (CI) are included between the brackets. The figure shows the sensitivity and specificity estimates for individual studies (squares) and 95% CIs (black horizontal lines).

individual studies stratified by specimen type. There was considerable heterogeneity within strata (for example, within the serum subgroup, sensitivity ranged from 28% to 100%). Estimated sensitivity ranged from 2% to 100%, and specificity from 33% to 100% across studies. Similar ranges of performance (sensitivity of 6% to 100% and specificity of 33% to 100%) were seen when the analysis was restricted to studies from LMIC (data not shown).

(ii) Urine LAM studies of HIV-infected and uninfected TB patients. As shown in Fig. 4, five studies reported the performance of urine LAM in individuals with TB-HIV coinfection; four of these studies reported data comparing LAM antigen detection in individuals with and without HIV infection. All four studies found improved sensitivity in HIV-infected compared with HIV-uninfected individuals (14% to 53% higher sensitivity), although in three studies, specificity was lower by 6% to 7%, and in one study, specificity was increased by 1%. In three studies involving HIV-TB-coinfected patients, results were stratified by CD4 cell count (20, 39, 67). All three studies found a higher sensitivity of urine LAM in patients with lower CD4 cell counts. We identified only one study that evaluated urine LAM in sputum smear-negative HIV-infected individuals. Sensitivity was 42%, with a 95% CI of 20 to 67%, and specificity was 84%, with a 95% CI of 68 to 94% (60).

Lawn et al. found that only 2 h were required for detection of LAM in urine compared with an average of 24 days for detection of *M. tuberculosis* complex by culture (39). In addition, five of 22 patients at risk for paradoxical TB immune

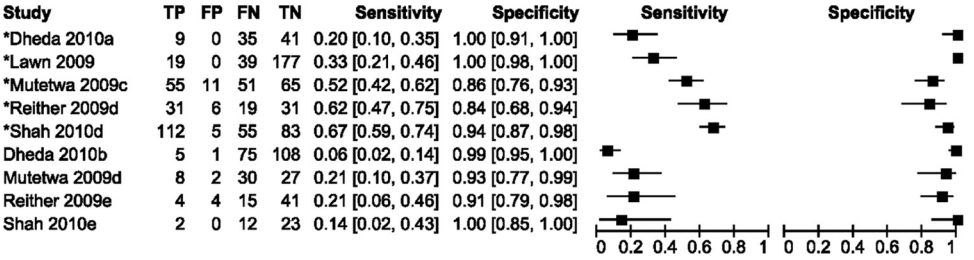

FIG. 4. Forest plots of sensitivity and specificity for urine LAM for pulmonary TB. Studies of HIV-infected patients are identified with "*." TP, true positive; FP, false positive; FN, false negative; TN, true negative. 95% confidence intervals (CI) are included between the brackets. The figure shows the sensitivity and specificity estimates for individual studies (squares) and 95% CIs (black horizontal lines).

reconstitution disease had detectable LAM. The authors noted a trend toward increased mortality in patients with LAM positivity, although this trend was not statistically significant (39).

(iii) Meta-analysis of studies of PTB. Among the 47 studies of PTB patients, there was considerable heterogeneity in specimen type, antigen identity, and smear and HIV status. We were able to identify five subgroups that were more homogeneous (Tables 3 and 4). For sputum LAM (studies of both smear-positive and -negative patients), the pooled sensitivity for LAM was 87%, with a 95% CI of 71 to 96%, and the pooled specificity 70%, with a 95% CI of 6 to 99% (Table 3). For urine LAM, the performances were similar in smear-positive (pooled sensitivity, 54%, and 95% CI, 18 to 86%; pooled specificity, 90%, and 95% CI, 83 to 95%) and smear-negative (pooled sensitivity, 51%, and 95% CI, 18 to 83%; pooled specificity, 90%, and 95% CI, 79 to 96%) patients (Table 4). Within studies of HIV-uninfected TB patients ($n = 4$), the pooled sensitivity of urine LAM was 14%, with a 95% CI of 4 to 38%, and the pooled specificity was 97%, with a 95% CI of 86 to 100%. In HIV-infected TB patients, the pooled sensitivity was higher but still low ($n = 5$, 47%; 95% CI, 26 to 68%), and pooled specificity similar (96%; 95% CI, 81 to 100%) (Table 5). The estimated probability that the pooled sensitivity of the test with HIV-infected patients exceeds that of the test with HIV-uninfected patients was 98%.

EPTB. (i) Characteristics of included studies. All 19 articles were written in English (4, 6, 13, 21, 29, 32, 34, 35, 47, 48, 52, 53, 56, 66, 68, 69, 82, 84, 87) (Table 5). The 21 EPTB studies involved 1,616 participants (sample size = 1,690). The majority (91%) of studies were conducted in LMIC. In four studies, HIV-infected individuals comprised 49% (21), 68% (52), 84% (53), and 85% (67) of the total enrolled patients. The remaining papers did not report HIV status. No studies involved children. In each study, all non-TB participants were from the same country as TB patients. The median number of TB patients included in each study was 22 (interquartile range of 14 to 31). Seventeen (81%) studies involved in-house tests, and four used the Clearview TB ELISA. Of the total 21 studies, 14 (64%) used the ELISA, 2 (9%) an agglutination test, and 5 immunohistochemical staining of biopsy specimens. In seven (33%) studies, the targeted antigen was unspecified.

Overall, studies had serious limitations: only three (14%) studies were considered to involve a representative patient population and only nine (43%) reported blinding of the antigen detection test result, (see Fig. S3 in the supplemental material). Figure 5 is a forest plot of the sensitivity and specificity of individual studies stratified by specimen type. There was considerable heterogeneity within strata (for example, within the biopsy specimen subgroup, sensitivity ranged from 36% to 100%). The estimated sensitivity for all studies ranged from 0% to 100%, and specificity from 62% to 100%. In the EPTB group, no studies reported patient-important outcomes.

(ii) Meta-analysis of studies of EPTB. Among the 21 studies of EPTB patients, there was considerable heterogeneity in disease site, specimen type, and antigen identity. We were able to identify three subgroups that were homogenous in terms of specimen type: serum, CSF, and biopsy specimen (Table 3). Studies targeting LAM, ESAT-6, Ag85 complex, and the 65-kDa antigen (65-kDa) in CSF, when pooled, yielded the highest sensitivity (87%; 95% CI, 61 to 98%), but low specificity (84%; 95% CI, 60 to 95%) (Table 3; Fig. 6).

TABLE 3. Pooled sensitivity and specificity estimates by subgroup, with posterior medians

Subgroup or specimen	No. of studies	No. of participants	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
Pulmonary tuberculosis				
Sputum lipoarabinomannan (LAM)	4	461	87 (71–96)	70 (6–99)
Extrapulmonary tuberculosis				
Serum[a]	4	246	76 (24–97)	89 (74–96)
Cerebrospinal fluid[b]	5	404	87 (61–98)	84 (60–95)
Biopsy/immunohistochemical staining[c]	4	174	80 (23–99)	86 (69–97)

[a] Antigens detected: excretory secretory (ES)-20 ($n = 1$); ES-31 ($n = 20$); EST-6 [contains 38 kDa (Rv0934) and 41 kDa], ES-20, and ES-31 ($n = 1$); the precise identities of ES20, ES-31, and EST6 are not known.

[b] Antigens detected: LAM ($n = 2$); Ag85complex (Rv3804c and Rv1886c, $n = 1$); ESAT-6 early secreted antigenic target-6 (Rv3875, $n = 1$); 65 kDa (Rv0440, $n = 1$). Pooled accuracy estimates are based on 5 studies with defined antigen targets. When data from an additional study targeting "nonspecific glycolipid antigens" were included (82), pooled sensitivity was 90% (56 to 99%) and pooled specificity 86% (72 to 94%).

[c] Antigens were unspecified in all studies.

TABLE 4. Pooled sensitivity and specificity estimates for urine lipoarabinomannan (LAM) detection[a]

Pulmonary TB subgroup	No. of studies	No. of participants	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
Smear-positive	6	906	54 (18–86)	90 (83–95)
Smear-negative	5	728	51 (18–83)	90 (79–96)
HIV-infected	5	844	47 (26–68)	96 (81–100)
HIV-uninfected	4	357	14 (4–38)	97 (86–100)

[a] Posterior medians with 95% credible intervals are given in parentheses.

DISCUSSION

Forty-seven PTB and 21 EPTB studies evaluating antigen detection tests for the diagnosis of TB were included in this systematic review. Eight subgroups comprising 37 studies were selected for meta-analysis. Within these subgroups, for both PTB and EPTB, sensitivity and specificity were variable. At a recent meeting convened by Médecins Sans Frontières, Partners in Health, and the Treatment Action Group, a multidisciplinary group of TB experts, including clinicians, test developers, laboratory experts, research scientists, and community representatives, proposed a set of minimal specifications for a point-of-care test based on modeling and expert opinion: in adults with pulmonary TB, the test should achieve a sensitivity of 95% in smear-positive and 60% to 80% in smear-negative cases and a specificity of 95%; and in extrapulmonary TB, the test should provide a sensitivity of 80% and specificity of 95% (41). In this review, for both PTB and EPTB, pooled accuracy estimates in all analyses fell short of achieving the required sensitivity and specificity values. However, we caution that our meta-analyses are based on a small number of studies, meaning that there is still considerable uncertainty in the usefulness or lack of usefulness of these tests. A new TB test may have several purposes, for example, triage, replacement test for microscopy, or add-on test following microscopy. Although we

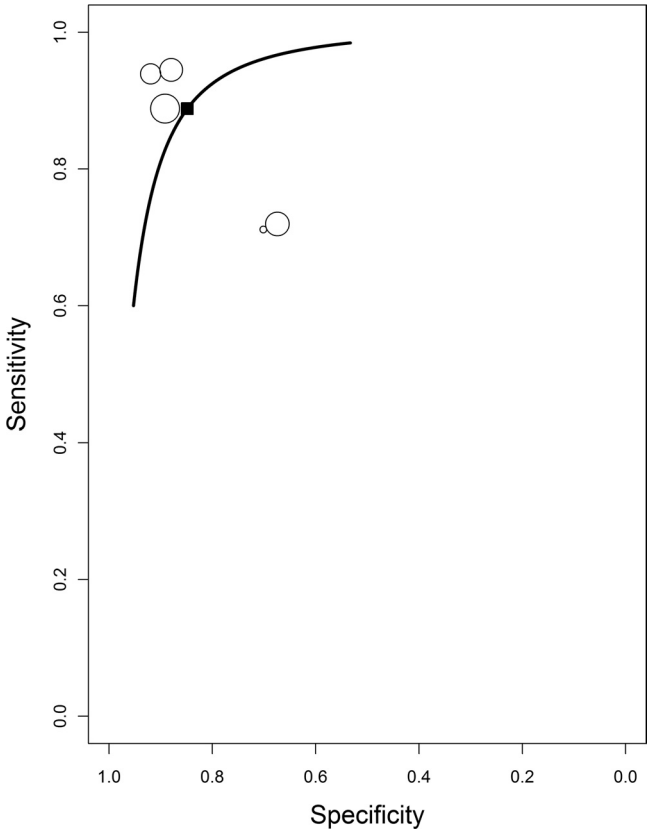

FIG. 6. Summary HROC plot of sensitivity and specificity for antigen detection tests of cerebrospinal fluid for the diagnosis of tuberculous meningitis. The width of the circles is proportional to the number of patients in each study. The square is the summary value for sensitivity and specificity.

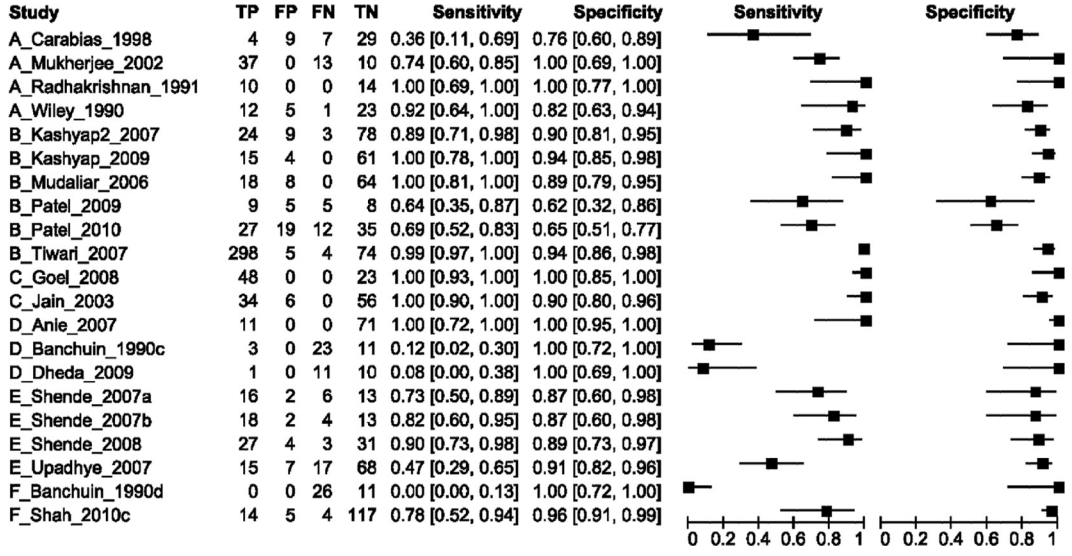

FIG. 5. Forest plots of sensitivity and specificity for antigen detection tests for the diagnosis of extrapulmonary TB, all studies. For letters preceding author names: A, biopsy specimen; B, cerebrospinal fluid; C, lymph node aspirate; D, pleural fluid; E, serum; F, urine. TP, true positive; FP, false positive; FN, false negative; TN, true negative. The 95% confidence intervals (CI) are included between the brackets. The figure shows the sensitivity and specificity estimates for individual studies (squares) and 95% CIs (black horizontal lines).

TABLE 5. Characteristics of included studies evaluating antigen detection tests for the diagnosis of extrapulmonary tuberculosis[a]

Specimen type	Author (reference) (year, study group)	Form of tuberculosis	Country	HIV status	Comparison group	Test name/antigen(s) detected (H37Rv designation)	No. of patients with/without TB	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
Biopsy									
	Carabias (13) (1998)	Multiple and unspecified	Spain	Not reported	OD	In-house/*M. tuberculosis* antigens, unspecified	11/38	36 (11–69)	76 (60–89)
	Mukherjee (48) (2002)	Lymph node	India	Not reported	OD	In-house/*M. tuberculosis* antigens, unspecified	50/10	74 (60–85)	100 (69–100)
	Radhakrishnan (56) (1991)	Meningeal	India	Not reported	OD	In-house/*M. tuberculosis* antigens, unspecified	10/14	100 (69–100)	100 (77–100)
	Wiley (87) (1990)	Not reported	USA	Not reported	OD	In-house/*M. tuberculosis* antigens, unspecified	13/28	92 (64–100)	82 (63–94)
Cerebral spinal fluid									
	Kashyap (34) (2007)	Meningeal	India	Not reported	OD	In-house/Ag85 complex (3804c and 1886c)	27/87	89 (71–98)	90 (81–95)
	Kashyap (35) (2009)	Meningeal	India	Not reported	OD	In-house/ESAT-6 (3875)	15/65	100 (78–100)	94 (85–98)
	Mudaliar (47) (2006)	Meningeal	India	Not reported	OD	In-house/65 kDa (0440)	18/72	100 (81–100)	89 (79–95)
	Patel (52) (2009)	Meningeal	South Africa	HIV±	OD	Clearview TB ELISA/LAM	14/13	64 (35–87)	62 (32–86)
	Patel (53) (2010)	Meningeal	South Africa	HIV±	OD	Clearview TB ELISA/LAM	39/54	69 (52–83)	65 (51–77)
	Tiwari (82) (2007)	Meningeal	India	Not reported	OD	In-house/glycolipid cocktail	302/79	99 (97–100)	94 (86–98)
Lymph node aspirate									
	Goel (29) (2008)	Lymph node	India	Not reported	OD	In-house/38 kDa (0934)	48/23	100 (93–100)	100 (85–100)
	Jain (32) (2003)	Lymph node	India	Not reported	OD	In-house/38 kDa (0934) and crude antigens	34/62	100 (90–100)	90 (80–96)
Pleural fluid									
	Anie (4) (2007)	Pleural	India	Not reported	ORD	In-house/tuberculosis-associated glycolipid protein	11/71	100 (72–100)	100 (95–100)
	Banchuin (6) (1990, c)	Pleural	Thailand	Not reported	ORD	In-house/PPD	26/11	12 (2–30)	100 (72–100)
	Dheda (21) (2009)	Pleural	South Africa	HIV±	ORD	Clearview TB ELISA/LAM	12/10	8 (0–38)	100 (69–100)
Serum									
	Shende (68) (2007, a)	Lymph node	India	Not reported	OD	In-house/ES-31	22/15	73 (50–89)	87 (60–98)
	Shende (68) (2007, b)	Lymph node	India	Not reported	OD	In-house/ES-20	22/15	82 (60–95)	87 (60–98)
	Shende (69) (2008)	Lymph node	India	Not reported	ORD	In-house/ES-20	30/35	90 (73–98)	89 (73–97)
	Upadhye (84) (2007)	Multiple*	India	Not reported	OD	In-house/ES-31, ES-43, EST-6	32/75	47 (29–65)	91 (82–96)
Urine									
	Banchuin (6) (1990, d)	Pleural	Thailand	Not reported	ORD	In-house/PPD	26/11	0 (0–13)	100 (72–100)
	Shah (66) (2010, c)	Disseminated	South Africa	HIV±	OD	Clearview TB ELISA/LAM	18/122	78 (52–94)	96 (91–99)

[a] ELISA, enzyme-linked immunosorbent assay; ES, excretory secretory [precise identity of antigen not known]; ESAT-6 (Rv3875), early secreted antigenic target-6; EST-6 contains 38 (Rv0934) and 41 kDa: LAM, lipoarabinomannan; OD, other diseases; ORD, other respiratory diseases; TB, tuberculosis; PPD, purified protein derivative; HIV±, people with and without HIV infection; *, includes lymph node ($n = 8$), meningeal ($n = 5$), bone and joint ($n = 6$), abdominal ($n = 5$), pleural ($n = 4$), and disseminated ($n = 4$) TB.

did not identify any studies that specifically considered the added value of antigen detection tests in addition to microscopy, in such a scenario, the required sensitivity for smear-positive patients may not need to be that great or necessarily higher than that for smear-negative patients.

LAM was the most frequently studied antigen, probably because it is a major constituent of the mycobacterial cell wall (12, 30), and a commercial LAM test has been developed. Although in this review the sensitivity of LAM detection was higher in sputum (87%) than urine (53%), urine LAM has important implications. First, it provides proof of principle that antigen detection with an easily obtained specimen can be exploited to develop a TB diagnostic test. Second, our meta-analysis demonstrates that urine LAM provides higher sensitivity in HIV-infected (47%) compared with HIV-uninfected (14%) TB patients, as was also found in a recent systematic review of urine LAM for TB (45). One possible explanation may be that the former set of patients has a higher or more disseminated bacterial burden with LAM antigenemia, leading to detectable LAM in urine. Of note, LAM positivity appeared to be inversely related to the CD4 count. Third, recent studies evaluating NAA tests with multiple specimens from the same patients have shown that this strategy increases the sensitivity of bacterial detection (59), suggesting that evaluation of both sputum and urine from the same patient may provide higher sensitivity than either specimen alone. Fourth, although in HIV-infected individuals we found the sensitivity of urine LAM for pulmonary TB to be relatively low, the combination of LAM testing and smear microscopy may provide additional benefit as proposed by Shah and colleagues (66). Although pooled specificity of urine LAM was relatively high (≥96%), specificity was found to be low (84% to 94%) in several studies and even lower in studies of serum and sputum LAM. Possible reasons include the presence of latent TB infection, misclassification of subclinical TB cases, or cross-reactivity with nontuberculous mycobacteria (3, 28, 30, 36). Studies are now required to clarify the cause of reduced LAM specificity.

For EPTB, the highest pooled sensitivity was achieved in tests for TB meningitis, a condition that is challenging to diagnose. Microscopy has poor sensitivity (~5%) for TB meningitis, and culture takes several weeks for results (80). Commercial NAA tests show high specificity (98%) and low and variable sensitivity (approximately 60%), require technical skill, and are expensive (51). With reported fatality rates above 50% (81), even a modest improvement in the accurate diagnosis of TB meningitis would be valuable. Two studies evaluating CSF LAM were well designed and reported (52, 53). Both involved a high proportion (>60%) of HIV-infected TB patients and achieved sensitivities of approximately 65%, suggesting that CSF LAM might be useful as a rule-in test for the rapid diagnosis of TB meningitis in high-HIV-prevalence settings. A point-of-care version of a LAM detection test for TB meningitis might be good to explore, especially if the test combined detection of LAM and other promising biomarkers, such as gamma interferon and adenosine deaminase (22, 83, 92).

Antigen detection tests for TB will require identification of antigens that have the following properties: abundant expression by *in vivo* bacteria, presence of antigens in the extracellular environment, and resistance to degradation by the enzymes that may be released during the inflammatory responses of the host. Moreover, antigens that are specific to *M. tuberculosis* will likely be necessary. Considering that *M. tuberculosis* adapts to its environment by modifying its transcriptional responses (25, 65, 78), it is not unreasonable to assume that the optimal antigens or antigen combinations for diagnosing different forms of EPTB may vary. Antigen detection in serum may be more difficult due to complex formation with the antibodies they elicit. The feasibility of using antigen detection tests for paucibacillary TB is unclear, but tests that detect multiple antigens may yield higher sensitivities than tests that detect single antigens. Besides LAM, no other antigens with potential value were identified in this review.

The strengths of our review include the use of standard guidelines, a comprehensive search strategy, two independent reviewers for all stages of the review process, and quality assessment with QUADAS. Although heterogeneity between diagnostic accuracy studies is to be expected (31), we attempted to address heterogeneity by prespecifying similar subgroups and by using a bivariate meta-analysis method.

Our review had limitations. The meta-analysis was limited by the small number of studies of any particular antigen detection test, with the exception of LAM. Our review did not account for different specimen processing techniques that may have contributed to variability in test performance. For example, 16 (80%) of the total 20 studies evaluating sputum used a processing method (studies differed with respect to the chemicals used and duration and gravitational force/speed of centrifugation), one study did not use a processing method, and three studies did not report whether or not a processing method was used. In studies evaluating urine LAM, two tests, the LAM-ELISA and the Clearview TB ELISA, were used. Although these assays contain the same polycolonal antibodies, the manufacturing processes differ; factors such as pH and viscosity may have affected diagnostic accuracy. In a systematic review of urine LAM for active TB, there was no statistically significant difference in performance noted between the groups of studies using fresh and previously frozen urine (45). In about 30% of the studies, test performance was reported, but antigen identity was unspecified. We were limited in our assessment of study quality by incomplete reporting of information. We encourage investigators to be more transparent about their studies. Publication bias is possible because studies with poor performance were unlikely to be unpublished. Statistical tests, such as funnel plots, are not recommended to detect potential publication bias in meta-analyses of diagnostic data (79). Accuracy estimates in our meta-analysis may be exaggerated because of the risk of bias from the selection of unrepresentative samples and lack of blinding (42, 62). The small sample size of individual studies contributes to heterogeneity between them. Going forward, researchers of primary diagnostic studies should follow published guidelines for conducting and reporting their work to ensure that their efforts contribute to a high-quality evidence base (7, 11).

In conclusion, because of the limited number of studies targeting any specific antigen other than LAM and concerns about methodological quality in a majority of studies, we could not draw firm conclusions about the clinical usefulness of antigen detection tests. Further studies are warranted to deter-

mine the value of LAM detection for TB meningitis in high-HIV-prevalence settings. Future studies are also needed that evaluate the performance of antigen detection tests in HIV-infected individuals with smear-negative TB. Considering that antigen detection tests could be translated into rapid and inexpensive point-of-care tests, research to improve their performance is urgently needed.

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Conflict of interests: K.R.S. serves as Coordinator of the Evidence Synthesis and Policy Subgroup of Stop TB Partnership's New Diagnostics Working Group (NDWG). A.R. served as the secretary of the NDWG (2006 to 2010) and is employed by WHO/TDR, the agency that administered the grant used to fund this systematic review. A.R. contributed to the conception and design of the systematic review and critical revision of and decision to publish the manuscript. J.M. is a recipient of the Quebec Respiratory Health Training Fellowship. M.P. is a recipient of a New Investigator Award from the Canadian Institutes of Health Research (CIHR). This funding source had no role in the preparation of the manuscript nor the decision to submit the manuscript for publication. M.P. serves as an external consultant for the Bill & Melinda Gates Foundation. M.P. also serves as a cochair of the NDWG. We have no financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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